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Orexin/Hypocretin: Wired for Wakefulness

Dispatch

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Orexin neurons play a crucial role in regulating wakefulness and energy metabolism, but until recently, little was known about the factors that influence the activity of these essential cells. Electrophysiological studies have now identified positive and negative feedback signals that allow the orexin neurons to help maintain wakefulness.

The survival of most animals relies upon maintaining alertness throughout their active period. When away from a safe sleeping spot, even a brief nap can be fatal. Recent electrophysiological studies have begun to identify how the neuropeptide orexin (also known as hypocretin) helps maintain wakefulness.

Orexin is produced by lateral hypothalamic neurons, which project widely throughout the brain, with especially heavy innervation of aminergic and cholinergic wake-promoting areas in the hypothalamus and brainstem [1–3]. Injection of orexin into the lateral ventricles increases wakefulness for hours [4,5], most likely because of its excitatory effects on these arousal regions. In mice, rats and dogs, a lack of orexin or dysfunction of the orexin receptors causes narcolepsy-like symptoms, such as frequent naps and sudden episodes of paralysis known as cataplexy [6–8]. In people with narcolepsy, the number of orexin-containing neurons is markedly decreased, and they too have great difficulty remaining awake across the day [9,10]. These consequences of orexin deficiency highlight the importance of this peptide in maintaining normal wakefulness.

Until recently, little was known about the factors that drive orexin neuron activity, but recent electrophysiological studies have identified several activators and inhibitors of the orexin neurons. By recording from hypothalamic slices of transgenic mice expressing green fluorescent protein (GFP) only in the orexin neurons, two groups [11,12] have shown that agonists of ionotropic glutamate receptors (AMPA and NMDA) excite orexin neurons, whereas glutamate antagonists (AP-5, CNQX or NBQX) reduce their activity. This excitation by glutamate persists during synaptic blockade, demonstrating that glutamate acts directly on the orexin neurons. These results clearly indicate that orexin neurons are tonically activated by glutamate, most likely released by nearby interneurons within the hypothalamic slice. Although orexin B peptide has little direct effect on the activity of orexin neurons, it increases this glutamate signaling [11]. Thus, neurons containing orexin and glutamate probably form a positive feedback loop that may reinforce and coordinate their activity (Figure 1).

In addition to this feedback loop, orexin neurons may be spontaneously active by virtue of their membrane properties. One group [13] found that the resting membrane potential of orexin neurons is close to their threshold for firing, even during synaptic blockade. This relative depolarization may be due to a calcium-activated, nonselective cation current that keeps these cells near their firing threshold. This propensity for spontaneous activity may allow the orexin neurons to remain persistently active, thus helping maintain wakefulness over long periods of time.

Wakefulness depends on the coordinated activity of many neuronal systems. The orexin neurons innervate and activate aminergic arousal regions such as the noradrenergic locus coeruleus, serotonergic raphe nuclei and histaminergic tuberomammillary nucleus [3]. Several researchers have hypothesized that amines should excite the orexin neurons, forming positive feedback loops that would maintain wakefulness [14], but these new electrophysiological studies show just the opposite. Both noradrenaline and serotonin hyperpolarize GFP-expressing orexin neurons [11,12]. Histamine has little effect on orexin neurons, but the histaminergic tuberomammillary neurons also release GABA [15], and GABA inhibits the orexin neurons [11–13].

It seems strange that wake-active aminergic areas would inhibit wake-active orexin neurons. Possibly, this noradrenaline and serotonin comes from aminergic nuclei other than the locus coeruleus and raphe nuclei which are not necessarily wake-active. Alternatively, the orexin neurons may become active late during the waking period, perhaps when aminergic regions are less active. For example, extracellular levels of noradrenaline and histamine rise just before the waking period and then gradually decrease over the next several hours [16,17]. In contrast, levels of orexin in rats and monkeys slowly increase across their waking period [18,19]. Each of these neurotransmitters may promote different aspects of wakefulness, especially as recent work indicates that orexin may counterbalance the rising sleep drive later in the day [19].

In addition to these synaptic pathways, peripheral humoral factors related to energy metabolism also influence orexin neurons. Starvation depletes fat stores and increases wakefulness in many animals. GFP-expressing orexin neurons are inhibited by leptin, a peptide produced by white adipose tissue [20]. During starvation, the orexin neurons may be disinhibited by low leptin levels. Conversely, orexin neurons are excited by ghrelin, a stomach-derived peptide which promotes feeding, and similar effects are seen with low levels of glucose [20]. These negative and positive signals may directly modulate orexin neuron activity according to appetite and bodily energy stores.

These new studies on the electrophysiology of the orexin neurons have substantially improved our

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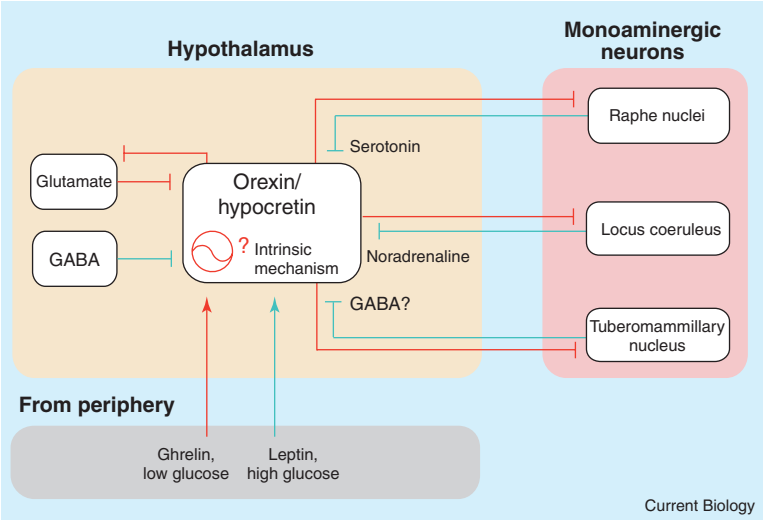

Figure 1. The activity of orexin/hypocretin neurons is regulated by positive and negative feedback loops.

Release of glutamate from hypothalamic neurons depolarizes orexin neurons directly. Orexin may activate these glutamate neurons, resulting in a positive feedback loop that reinforces their activity. Sustained activity of orexin neurons may also result from an intrinsic mechanism that keeps the orexin neurons relatively depolarized. Although orexin excites aminergic brain regions, noradrenaline and serotonin hyperpolarize orexin neurons. Histaminergic tuberomammillary neurons might participate in this negative feedback system by releasing GABA. These negative feedback loops may allow orexin neurons and aminergic arousal systems to be maximally active at separate times. Peripheral indicators of energy balance, such as glucose, ghrelin, and leptin, also directly influence orexin neurons, perhaps helping promote wakefulness during starvation. Red lines, excitatory signaling; blue lines, inhibitory signaling.

understanding of the pathways that govern sleep and wakefulness. Positive feedback loops between orexin and glutamate neurons as well as a tendency towards spontaneous activity may allow the orexin neurons to remain active, even as sleep drive rises over the day. In addition, the modulation of these neurons by signals related to appetite and energy balance may help explain why it is difficult to sleep when hungry. These properties should allow the orexin neurons to promote alertness in a hungry animal and maintain long periods of wakefulness across the day.

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